cannot be produced by chemical synthesis. Applicants contend that the term "synthetic inhibitor of urokinase" is not intended to define a pharmacologic classification of WX-UK1. The fact that a substance inhibits the enzyme urokinase in *in vitro* tests, does not support an assumption that this substance is principally pharmaceutically and therapeutically suitable. To enable a pharmacological classification of a given substance, the substance has to fulfill a series of characteristics, for example, with regard to toxicity, bioavailability, pharmacokinetics, biodistribution, metabolism, teratogenicity. These characteristics, defining the actual pharmacological suitability *in vivo,* cannot be derived by the skilled artisan from the mere fact that a test substance *in vitro* shows an inhibitory effect on the target enzyme urokinase.

Applicants also contend that contrary to statements in the Office Action, the publication Xing cannot be considered as a disclosure for the general use of urokinase inhibitors as antitumor agents alone or in combination with other active substances. Since the pharmacological applicability of a given substance has to be determined by a number of specific characteristics as explained above, the suitability of a specific urokinase inhibitor (B-428) as antitumor agent, does not implicitly suggest a therapeutic use for any and all other substances showing an *in vitro* inhibiting activity against urokinase. Applicants point out that the well known substance Amiloride is a very efficient urokinase inhibitor. However, Amiloride is not pharmacologically useful due to its antidiuretic side effect and thus is not suitable as an antitumor agent. Though there are hundreds of urokinase inhibitors, only a small number of urokinase inhibitors are pharmacologically interesting and have the potential to be developed further as therapeutics. There are many substances which show a urokinase inhibiting effect *in*

vitro, but are not suitable for pharmacological and therapeutic uses.

Rockway and Giranda, Cur Pharm Design, 2003, 9, 1483-1498 (copy attached), presents all chemical classes of urokinase inhibitors which are currently known, and concludes in the summary that most of the known urokinase inhibitors are not suitable to enter preclinical trials, or clinical studies *in vivo.* In other words, most of the known urokinase inhibitors are not pharmacologically useful (page 1494, right column, 4th paragraph to page 1495, left column, 2nd paragraph). In particular Rockway and Giranda allege that the urokinase inhibitors Amiloride (p. 1483, right column, last paragraph) and B-428 (page 1484, left column, 2nd paragraph), showed contrary results in studies on experimental tumors. In both cases, even though potency and selectivity of the urokinase inhibitors was shown *in vitro,* in different cancer models it appeared that they did not have any effect on the growth or metastatic spread of the tumors or produced very modest tumor growth reduction. The authors came to the conclusion that no clear statement is possible as to whether the examined urokinase inhibitors are in fact usable as an antitumor agent and/or whether the antitumor effect in question can in fact be related back to the inhibitors of the urokinase. Thus, this reference indicates that general statements cannot be made regarding the potential effect of urokinase inhibitors as pharmaceutical agents based on in vitro results.

As further support for the argument that not every urokinase inhibitor shows *in vivo* antitumor activity, applicants point out the abstract Pilat et al., Oncol Rep, 1998, 4, 889-892 (copy attached) which indicates that the urokinase inhibitor Amiloride was ineffective as an antitumor agent. In particular Amiloride did not inhibit tumor growth or metastases development in the rat prostate cancer model.

Steinmetzer, Idrugs, 2003, 6, 138-146 (copy attached), summarizes the state of the research of urokinase inhibitors prior to 2003. Amidinothiophene derivatives (page 142, left column, 1st paragraph) were discussed as having *in vitro* antimetastatic activity but the very low solubility of these derivatives would be an obstacle to the development of this substance group as an antitumor agent and thus they are not pharmacologically usable. In the conclusion it is further stated, that new lead compounds had been identified, however, the individual suitability as an antitumor agent, as well as the suitable bioavailability and elimination half-life, must still be determined by further preclinical studies (page 144, right column, 3rd paragraph).

In view of the above discussion, applicants contend that a multitude of urokinase inhibitors exist which are used *in vitro* for research purposes, especially as *in vitro* reagents in the research of the biologic and medical role of urokinase. However, the explicit examples of Amiloride and B-428 clearly prove that despite the ability of these compounds to inhibit urokinase *in vitro,* in several tumor models these compounds did not prevent the growth and the spreading of malignant tumors. Thus, it was not obvious at the time of the present invention that urokinase inhibitors per se must be regarded as antitumor agents. Applicants contend that the use of the claimed substance Na(2,4,6-triisopropylphenylsulfonyl)-3-amidino-(L)-phenylalanine-4-ethoxycarbonyl-piperazide (WX-UK1), as a pharmacologically active agent and the determination that the claimed compound prevents the growth and metastasis of tumors *in vivo,* would not have been obvious over the combination of the Pentapharm product catalog, Xing, De Vita and Medenica. The inventors of the present invention have shown that Nα(2,4,6-Triisopropylphenylsulfonyl)-3-amidino-(D,L)-phenylalanine 4-ethoxycarbonylpiperazide

is not hypothetical but in fact suitable to be used as a pharmacological active anti-cancer agent. The efficiency against the growth and the metastases of tumors *in vivo* has been proven and also the bioavailability and the low toxicity as explained by the corresponding assays in the Examples of the present application (cf. Examples 5 to 11).

The present invention does not claim the combination of any and all urokinase inhibitors with other antitumor agents, but the specific combination of the urokinase inhibitor Nα(2,4,6-Triisopropylphenylsulfonyl)-3-amidino-(D,L)-phenylalanine 4-ethoxycarbonylpiperazide with cytotoxic antitumor agents such as platinum derivatives, 5-fluoruracil derivatives and taxanes. The only cited reference which discloses Nα(2,4,6-Triisopropylphenylsulfonyl)-3-amidino-(D,L)-phenylalanine 4-ethoxycarbonylpiperazide is Pentapharm which discloses the compound as a research chemical not a therapeutic agent. None of the remaining references cures this deficiency as none of the other references indicate that in vitro results are predictive of in vivo effects for all urokinase inhibitors. In view of the above discussion regarding the unpredictability of in vivo activity based on in vitro results, applicants request that this rejection be withdrawn.

Applicants respectfully submit that all of claims 1-17 are in condition for allowance. If it is believed that the application is not in condition for allowance, it is respectfully requested that the undersigned attorney be contacted at the telephone number below.

In the event this paper is not considered to be timely filed, the Applicant respectfully petitions for an appropriate extension of time. Any fee for such an extension together with any additional fees that may be due with respect to this paper, may be charged to Counsel's Deposit Account No. 02-2135.

Respectfully submitted,

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